Assiut Vet. Med. J. Vol. 41 No. 82, July 1999

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COMPARATIVE LEVELS OF RACHIDONIC ACID AND EICOSAPENTAENOIC ACID IN EGYPTIAN FISH

(With 3 Tables)

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(Received at 5/6/1999)

النسب المقارنة لحمض الأراكيدونك والإكوسابنتاإنويك فى السمك المصرى

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تم إجراء دراسات على الأحماض الدهنية الغير مشبعة الداخلة فى تخليق الفوسفوليبيدات لمعظم الكائنات البحرية (أسماك الماء العذب والمالح) لما لهذه الأحماض من أهمية فى المحافظة على الصفات الطبيعية لجدر الخلايا. وقد لوحظ وجود خلافات هامة فى نسب الأحماض الدهنية الغير مشبعة (Omega-3) بين أسماك الماء العذب والمالح. كذلك وجد أن نسب حمض الأراكيدونك (٤:٢٠) واللينوليك (٢:١٨) كانت أعلى فى معظم الأسماك الموجودة فى الماء العذب عنهما فى أسماك الماء المالح ، بينما لوحظ أن هناك نسبة عالية فى الأحماض الدهنية الإكوسابنتاإنويك (EPA ٥:٢٠) والدكوساهيكسانويك (DHA ٦:٢٢) فى لحوم السمك الذى يعيش فى الماء المالح ، أيضا وجد أن النسبة بين الأحماض الدهنية (n-3/n-6) و ($C_{18:1}/C_{18:2}$) كانت أعلى فى أسماك الماء المالح عنها فى أسماك الماء العذب. ولذا ننصح بتناول أسماك الماء المالح بكميات كبيرة وبانتظام لما لدهون هذه الأسماك من فائدة كبيرة لصحة الإنسان والوقاية من أمراض القلب والسرطان والسمنة.

SUMMARY

Studies were done on the phospholipid fatty acid composition of the most important aquatic animals flesh which represents the freshwater fish and marine fish. In these species, important differences were discovered in the quantitative composition of the omega-3 fatty acids. The present data give an evidence that the freshwater fish contains higher levels of arachidonic (AA, 20:4 n-

4) and linoleic (18:2 n-6) acids than those in marine fish. However, in marine fish, n-3 fatty acids, particularly docosahexaenoic (DHA, 22:6 n-3) and eicosapentaenoic (EPA, 20:5 n-3) acids were the dominant unsaturated fatty acids, while the n-6 fatty acids (20:4 n-6, 18:2 n-6 and 22:5 n-6) acids were found in low levels. The ratio of n-3/n-6 and $C_{18:1}$/ $C_{18:2}$ were much higher in marine fish than those in freshwater fish. So we may concluded that the beneficial effect of fish oils especially marine fish is attributed mainly to the large amount of 22:6 n-3 and 20:5 n-3 fatty acids which reduce the risk of cardiovascular diseases, cancer and obseity.

Key words: *Fresh-water fish, marine fish, n-3 fatty acids*

INTRODUCTION

The nutritional properties of long chain n-3 fatty acids, especially eicosapentaenoic (EPA, 20:5 n-3) and docosahexaenoic (DHA, 22:6 n-3) acids, have been under intensive study in the last decade. These fatty acids seem to be able to modify lipid metabolism, membrane properties, platelet behavior etc. (Kinsella, 1988). Fish is the source of EPA and DHA in human nutrition.

On the other hand, the lipids of marine and freshwater fish have ertain characteristic differences in the fatty acid composition (Hilditch and William, 1964). These distinctions are based mainly on the chain lengths rather than any other fundamental properties (Ackman, 1967).

The most characteristic features in freshwater fish are the resence of C_{16} and C_{18} fatty acids in higher levels, while the fatty acids C_{20} and C_{22} are found in lower levels compared to those in marine fish (Ackman, 1967; Yamada and Hayashi 1975). These differences may be attributed to dietary fatty acids variations (Fogerty et al., 1986).

The fatty acid compositions of common fish in the northern emisphere are all well known, and they are also the main sources for the fish oil industry (Young and Chem, 1989). Nowadays the inclusion of fish in the diet is the only safe way to increase the intake of these fatty acids (Venkatraman et al., 1992). Ackman (1989), reported that many fish from warm and temperate water contain considerable of n-6 fatty acids.

The data about the fatty acid composition especially n-3 and n-6 ones in Egyptian marine and freshwater fish has been quite fragmentary until now which motivated to the present study.

MATERIAL and METHODS

Animals:

The following animals were used in this study:

Collecting centers:

1- In Egypt, there are three main regions for collecting marine fish, all are located along the coastal line of Mediterranean around or near by lake mouths or river mouths. These regions are namely, Mex, Rosetta and Damietta.

2- For the experiments, fish were collected from their natural habits, the water temperature varied according to the season. The three pelagic Sardines spp. were caught by purse-serine.

Lipid analysis

Extraction of total lipids:

Lipid extraction was performed according to Folch et al., (1957) by homogenizing the tissue in an all glass potter homogenizer in the presence of ice cold chloroform-methanol 2:1 containing 0.01% butylated hydroxytoline (BHT).

Phospholipids were separated by silicic acid column chromatography using chloroform to remove the neutral lipids and methanol to obtain the phospholipid fractions. The polar head group composition of the latter was determined according to Rouser et al., (1970).

Total phospholipids were transmethylated in the presence of 5% HCl in absolute methanol at 80°C in sealed vials for 2.5 hrs.

Gas Chromatography of fatty acid methyl esters:

Methyl esters were separated using a Hewlett-Packard 5890 II equipped with a capillary column coated with SP 2330 of 0.25 m thickness (0.25 mm I. D. x 30 mi CPS-Li Quadrex, New Haven, CT,U.S.A.). High-purity nitrogen was applied as carrier gas with a flow rate of 230 KPa. Hydrogen was used at 100 KPa and 280 KPa. The dual column system was programmed from 160°C to 200°C to give partial separation of 18:3 (n-3) and 20:1 (n-9) at a rate of 1°C/min. The detector temperature and injector temperature were 250°C and 230°C, respectively. The peaks were identified by means of primary and secondary standards and by plotting log relative elution temperature

versus the number of carbon atoms (Schmit and Wynner, 1966). The percentage composition was calculated as a weight percentage (w/w %) using a Hewlett-Packard 3396 A integrator. All peaks between myristic acid (14:0) and DHA (22:6 n-3) were included in the calculations.

RESULT and DISCUSSION

The fatty acid compositions of freshwater and marine fish are shown in (Table 2 and 3), for the different species studied. The values are given as weight percent of total fatty acid methyl esters. Fatty acids are designated by number of carbon atoms, and number of double bonds. The digit after n states the number of carbon atoms from the methyl end of the acyl chain to the nearest double bond.

Common names are not indicated because fatty acids in fish are primarily unsaturated and they are mixtures of several isomers (Hearn et al., 1987), that is the double bonds are located in different positions. Only those fatty acids that were consistently detected at the level of 0.5% or more of the total are given in (Table 2 and 3) which also give the amount of polyunsaturated fatty acids (PUFAs) of n-3 and n-6 families. This amount is the sum of all PUFA belonging to the same family: 18:4, 20:5, 22:6 of the n-3 series and 18:2, 20:2, 20:3, 20:4, 22:4 and 22:5 of the n-6 series, respectively.

The ratio of n-3 over n-6 is given for comparative purpose with regard to n-6 PUFA species such as Tilapia nilotica, Pike, Cat-fish, Bagrus-bayad and denees. Freshwater fish contained more than 15% of the total acids. The major n-6 fatty acid was 20:4 other fatty acids of the n-6 series present include linoleic (18:2 n-6), docosatetraenoic (22:4 n-6) and docosapentaenoic (22:5 n-6).

With regard to n-3 PUFA, marine fish all contained more than 40% n-3 PUFA. Carp, Silver carp and Catfish were lowest in percentage of n-3 PUFA, all had 11.42, 11.98 and 8.5% respectively. The ratio of n-3/n-6 PUFA was nearly one for Pilchard and Sea-bass marine fish.

Docosahexaenoic (DHA, 22:6 n-3) was clearly the most abundant of PUFAs in all marine species. The rest of the n-3 fatty acids was mostly composed of eicosapentaenoic (EPA, 20:5 n-3) and docosapentaenoic acid (22:5 n-3).

The ratio of n-3/n-6 and 18:1/18:2 were found to be higher in marine fish than those in fresh water fish.

The proportion of total monounsaturated acids is lower in marine lipids and higher in fresh-water lipids. Values for total saturated and total

polyunsaturated fatty acids show much scattering, like fish from tropical waters (Nair and Gopakumar, 1978). There are considerable differences between the fish species, reflecting the differences in higher diets (Cai and Curtis, 1990; Rady et al., 1993).

Recently omega-3 fatty acids have been the focus of much attention. These fatty acids may be incorporated into membranes where they many affect phospholipid fluidity, molecular packing and activity of membranes bound enzyme or they may be involved in the production of prostaglandin E_2, thromboxan A_2, prostacyclin and leukotrienes, (Ehringer et al., 1990). Many factors may be the cause of important differences in fatty acid composition between marine and fresh water fish. Korshom et al. (1994) found that the blood chemistry are influenced by species differences and storking system.

On the other hand, Fogerty et al. (1986) concluded that the fatty acids of some marine fish are principally of dietary origin. Furthermore, Rady et al. (1993) found a correlation between the fatty acids composition of feed and lipid metabolism of the fish. The obtained data (Table 2) proved that marine fish contain high levels of n-3 fatty acids and low concentrations of arachidonic acid. Fischer et al. (1986) and Simopoulos (1991) reported that the incidence of myocardial infraction and coronary atherosclerosis among Greenland Eskimos is low, since they are consume significant amounts of marin fish on a regular basis, throughout their life.

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Table 1.

Animal Species	Common name	Scientific name	Type of diet	Origin
Freshwater Fish	Tilapia spp. Nile tilapia Cyprineda Common carp Silver carp Grass carp Sharptooth Cat_fish Bagrus bayad Eel (Hamash) Pike Denees	Oreochromis niloticus Oreochromis aureus Cyprinus carpio Hypophthalmichthys molitrix Chenopharyngodon idellus Clarias gariepinus Clarias lazera Anguilla anguilla Esox lucius L. Sparus aurata	Benthic algae Benthic feeder on algae and detritus Benethic feeder Phytoplankton Omnivorous but mostly a predator Predator carnivorous Predator carnivorous Small fishes Small Crustaceans and Molluscs	Nile River at Behera Edffina
Marine fish	Gilt – Sardine Pilchard Sea bream Sea bass Sole (Mousa) Grey mullets	Sardinalla aurita Sardina pilchardus Dicentrachus Labrax Solea solea Mugil cephalus	Benethic on Algae and Diatomes Small fishes Crustaceans and Molluscs Diatomes Algea	Mex Alex.

Table 2.

Fatty acid composition of marine fish flesh

Fatty acids %	Gilt sardine	Pilchard	Sea bream	Sea bass	Grey mullets	Sole
14:0	3.4	3.35	0.50	1.49	2.98	3.93
15:0	1.3	1.93	--	--	0.71	1.88
16:0	14.52	15.11	22.10	19.30	13.47	11.24
16:1 n-7	7.09	5.89	2.15	4.94	5.99	9.79
18:0	4.93	10.77	4.79	3.73	5.18	4.24
18:1 n-9	15.41	16.99	9.48	18.20	18.21	13.78
18:2 n-6	1.39	1.67	1.19	2.21	1.67	2.67
18:3 n-3	--	--	--	--	0.60	--
18:4 n-3	3.82	--	--	2.75	2.24	3.49
20:1	4.24	2.45	2.12	2.92	8.15	5.91
20:2 n-6	--	--	0.82	--	--	--
20:3	--	--	--	--	--	--
20:4 n-6	0.90	1.88	1.54	2.11	2.14	2.20
20:4 n-3	--	--	--	--	--	--
22:1	1.34	--	2.09	--	--	1.97
20:5 n-3	11.32	8.31	16.28	15.31	14.51	15.80
22:4 n-6	0.75	--	--	--	2.91	--
22:5 n-6	0.61	--	--	--	1.11	--
22:5 n-3	2.51	4.10	0.69	1.36	2.69	--
22:6 n-3	25.79	25.44	36.12	25.64	17.37	23.00
n-3	43.40	37.85	53.10	45.06	36.81	42.29
n-6	3.70	3.55	2.78	4.32	8.43	4.87
n-3/n-6	11.73	10.66	19.11	10.43	4.37	8.68
18:1/18:2	11.09	10.17	7.97	8.25	10.90	5.16

Table 3.

Fatty acid composition of fresh-water fish flesh

Fatty acids	Common carp	Grass carp	Silver carp	Tilapia nilotica	Tilapia zilli	Eel	Pike	Cat-fish	Bagrus-bayad	Denies
14:0	1.73	1.45	2.94	4.25	2.75	4.99	3.51	1.91	3.71	1.63
15:0	–	0.61	1.38	0.99	1.00	–	1.73	–	3.87	1.51
16:0	18.48	22.51	19.48	16.65	22.01	19.51	7.23	21.52	12.58	15.98
16:1 n-7	10.98	8.34	9.58	11.07	5.83	12.86	6.24	4.73	9.76	3.08
18:0	5.54	4.52	4.78	8.71	7.92	1.97	4.19	6.98	10.78	10.47
18:1 n-9	31.97	25.40	27.08	21.47	22.42	38.25	7.65	29.85	12.97	14.08
18:2 n-6	13.34	11.98	10.36	2.83	7.91	4.11	3.81	7.32	5.19	1.78
18:3 n-6	0.81	–	–	–	1.56	–	–	–	–	–
18:4 n-3	–	–	–	1.45	0.41	–	–	–	1.63	–
20:1	–	2.98	3.18	3.79	1.33	2.94	13.78	3.87	1.89	2.28
20:2	–	–	–	–	1.29	–	–	1.76	0.98	0.61
20:3	–	–	–	0.58	1.11	–	–	–	2.99	0.99
20:4 n-6	5.73	6.44	5.95	6.04	6.02	1.98	18.98	3.11	6.57	13.34
20:4 n-3	–	–	–	–	0.25	–	2.31	1.23	–	–
20:5 n-3	6.88	7.02	5.51	2.18	4.08	1.7	7.98	5.01	10.89	9.86
22:4 n-6		0.24	0.69	–	–	–	1.79	–	0.91	3.32
22:5 n-6		–	–	1.91	–	–	–	–	0.97	1.98
22:5 n-3		2.07	2.39	4.87	2.94	4.83	3.26	2.34	6.11	5.41
22:6 n-3	4.54	6.43	6.47	13.13	9.11	6.85	17.53	10.37	8.19	13.67
n-3	11.42	14.57	14.37	24.57	16.79	13.38	31.08	18.95	26.82	28.94
n-6	19.88	18.73	17.0	11.36	14.56	6.09	24.58	12.19	17.62	22.02
n-3/n-6	0.57	0.78	0.84	1.90	1.15	2.20	1.26	1.55	1.52	1.31
18:1/18:2	2.4	2.12	2.61	7.59	2.83	9.31	2.01	4.08	2.5	7.91